

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.

APPENDIX B

Matrix metalloproteinase degradation of extracellular matrix: biological consequences

Steven D Shapiro

Targeted mutagenesis has allowed investigators to perform controlled experiments in mammals and determine the contribution of individual proteins to physiologic and pathologic processes. Recent lessons learned from matrix metalloproteinase gene targeted mice and other *in vivo* observations have given new life to old concepts regarding the role of proteolytic fragments of extracellular matrix proteins in regulating a variety of critical processes in cell biology.

Addresses

Departments of Pediatrics, Medicine and Cell Biology and Physiology, Washington University School of Medicine at Barnes-Jewish Hospital, (North Campus), 216 South Kingshighway, St Louis, MO 63110, USA; e-mail: sshapiro@imagine.wustl.edu

Current Opinion in Cell Biology 1998, 10:602-608

<http://biomednet.com/elecref/0855067401000602>

© Current Biology Ltd ISSN 0955-0674

Abbreviations

ADAM a disintegrin and metalloproteinase domain
APC adenomatous polyposis coli
ApoE apolipoprotein E
ECM extracellular matrix
LLC Lewis lung cell carcinoma
MMP matrix metalloproteinase
PGK phosphoglycerol kinase
TIMP tissue inhibitor of metalloproteinases
TNF tumor necrosis factor α
t-PA tissue-type plasminogen activators
u-PA urokinase-type plasminogen activators

Introduction

Matrix metalloproteinases (MMPs) comprise a family of extracellular matrix degrading enzymes (Table 1) that are believed to play pivotal roles in embryonic development and growth as well as in tissue remodeling and repair. Excessive or inappropriate expression of MMPs may contribute to the pathogenesis of many tissue-destructive processes, including highly prevalent diseases such as arthritis, multiple sclerosis, and tooth decay, as well as the leading causes of death in developed countries: cardiovascular disease (atherosclerosis plaque rupture and aneurysm formation), tumor progression, and chronic obstructive pulmonary disease¹. A statement such as this is usually found at the beginning of MMP-related papers (and of course grant applications — simply insert disease of interest) written by those of us with a 'metallocentric' view of the world [1*]. But judgment day is approaching. With the advent of targeted mutagenesis, one can perform controlled experiments in mammals to test these hypotheses. Moreover because these important diseases can potentially be attributable to the action of MMPs, effective synthetic MMP inhibitors are being developed and are rapidly approaching clinical trials [1*,2]

Targeted mutagenesis of MMPs

Over the past couple of years, many of the MMPs have undergone gene-targeting experiments (Table 2). The power of gene targeting was best summarized by Piagen, who stated, "one invariable lesson of biological research has been the difficulty, virtual impossibility, of reliably predicting the properties of intact organisms from the properties of their constituent tissues, cells and molecules. Thus, hypotheses need to be confirmed in intact, complex biological organisms; not prokaryotes or lower eukaryotes, but mammals [3]." One must, of course, recognize the limitations of these experiments. First, loss of a protein from the blastocyst stage onward might alter complex biological processes, leading to what is commonly referred to as compensation. Second, because of gene redundancy, mutation of a gene may not unmask the true biological function of the protein it encodes. Third, mice are not humans; hence, direct translation of results to human biology requires knowledge of biological similarities and differences between these species [4].

When interpreting data from gene-targeting experiments one must also be aware of potential strain differences and the possibility of 'neighborhood knock-out' effects. This term refers to inhibited expression of genes physically linked to the target gene, and is probably related to the retention of the phosphoglycerol kinase (PGK) promoter used to drive selectable markers [5]. This is a pertinent concern because several MMP genes (collagenases, stromelysin, matrilysin, and macrophage elastase) are closely linked on human chromosome 11q22 and mouse chromosome 9. With these caveats in mind, what have MMP-mutant mice told us about the biological consequences of MMP-mediated extracellular matrix (ECM) degradation?

Physiological processes

MMPs, usually undetectable in cells under normal circumstances are prominently expressed during a variety of biological processes, such as reproduction. On the maternal side, MMP expression is associated with menstruation, ovulation, uterine implantation, parturition, and postpartum uterine and mammary gland involution [6]. From the offspring's perspective, MMPs are believed to be required for trophoblast implantation, embryonic growth, and tissue morphogenesis. Yet, none of the individual MMP-mutant mice generated to date have had an embryonic lethal phenotype. Mice deficient in gelatinase B (MMP-9^{-/-}) demonstrate morphologic abnormalities at the site of implantation, but these defects are not lethal. All MMP-deficient mice to date are capable of delivering and nurturing healthy pups.

Table 1

Matrix metalloproteinase substrates^a.

MMP	Interstitial collagens	Basement membrane	Elastin	Non-matrix proteins
Collagenases				
MMP-1	III > I > (+/- II); VII, X	+/- FN, LN, EN, PG	-	L-selectin is a substrate for collagenases
MMP-8	I > III > II; (? VII, X)	+/- FN, LN, EN, PG	-	
MMP-13	II > I, III, GL (? VII, X) and telopeptidase	+/- FN, LN, EN, PG	-	
Stromelysins				
MMP-3	-	FN, LN, EN, PG, +/- PS col IV	+/-	EGF-like growth factor and plasminogen are substrates for stromelysins
MMP-10 ^a	-	FN, LN, EN, PG, +/- PS col IV	+/-	
Stromelysin-like				
MMP-7	-	FN, LN, EN, PG	+	Stromelysin-like enzymes are most potent at converting plasminogen to angiotensin and degrading α_1 AT
MMP-12	-	FN, LN, EN, PG, PS col IV	++	
Gelatinases				
MMP-2	GL, I, VII, X, XI	col IV/V, FN, LN, EN, PG, PS	++	
MMP-9	GL	col IV/V, FN, LN, EN, PG, PS	++	
Furin-recognition sites				
MMP-11 ^b	-	-	-	
Membrane type				
MMP-14 ^c	+/- I > III, II	FN, LN, EN, PG		
MMP-15		FN, LN, EN, PG		
MMP-16	?			
MMP-17	?			
Newly described				
Enamelin	GL (amelogenin)	?	?	?

^aNote this list is inherently incomplete, representing only selected substrates tested to date. These substrates guide potential biological functions, but the actual *in vivo* substrates are unknown. ^bMMP-10 has the same substrate specificities as MMP-3 but is less potent. ^cHuman

enzyme not catalytically active to known ECM components. ^dSoluble recombinant protein was tested. α_1 AT, α_1 antitrypsin, EN, elastin; FN, fibronectin; GL, gelatin; col IV/V, types IV and V collagen; LN, laminin; PG, proteoglycan; PS col IV, pepsinized type IV collagen.

Post-natal development

The major defect in MMP-9^{-/-} deficient mice is delayed long bone growth and development [7^{*}]. Long bones develop from mesenchymal condensations where cartilage cells differentiate and deposit a cartilage matrix. Blood vessels invade and degrade the cartilage matrix, and cartilage cells undergo apoptosis followed by proliferation of osteoblasts and endochondral ossification, which converts the tissue into mature bone. In MMP-9^{-/-} mice, there is delayed vascular invasion of skeletal growth plates, resulting in an excessively wide zone of hypertrophic cartilage and delayed ossification. MMP-9 is required to initiate primary angiogenesis in the cartilage growth plate, probably through generation of an angiogenic signal (or perhaps degradation of an angiogenesis inhibitor). Interestingly, the mechanism of this phenotype may not involve degradation of a structural or adhesive matrix protein.

While this phenotype is marked during growth, if one were merely to study adults only a 10% shortening in the long bones would be appreciated. This is not meant to diminish the importance of this finding but, rather, it emphasizes that until careful analyses of all MMP^{-/-} mice

are performed conclusions regarding the role of individual MMPs in growth and development are premature. The overall minimal phenotypes observed to date may be due to redundancy, safeguarding the host from untoward consequences of individual MMP mutations. Generation of doubly and multiply MMP deficient mice may be required to unmask full MMP function. Alternatively, MMPs may not be needed for grossly normal development and growth in the mouse.

MMP-9 was demonstrated in the mesenchyme of embryonic kidneys, and branching morphogenesis of metanephric buds was specifically blocked in metanephric organ culture by antisera to MMP-9 but not by IgG antibodies to MMP-2 [8]. No abnormalities were found upon analysis of neonatal and adult MMP-9^{-/-} mice by light microscopy or immunofluorescence for basement membrane proteins, however, and renal function in adult mice was normal [9]. It is not clear whether the discrepancies between these studies result from differences in study design, *in vivo* versus *in vitro* studies, or whether antibody experiments overestimate the consequences of gene inactivation while gene knockout experiments underestimate them.

Table 2

Phenotypes of MMP-deficient and related null mutant mice.

Mice	Result	Reference
MMP-deficient		
Goldfinch A (MMP-2)	Unaltered secretion of β -amyloid precursor protein	[49]
	Reduced angiogenesis and tumor progression	[21]
Stromelysin-1 (MMP-3)	No effect on collagen-induced arthritis	[50]
Matrikysin (MMP-7)	Decreased intestinal tumorigenesis	[22*]
Gelatinase B (MMP-9)	Impaired primary angiogenesis in bone growth plates	[7*]
	Resistant to bullous pemphigoid	[41]
Stromelysin 3 (MMP-11)	Decreased chemical-induced mutagenesis	[23*]
Macrophage elastase (MMP-12)	Impaired macrophage proteolysis	[14]
	Impaired macrophage recruitment and protection from cigarette-smoke-induced emphysema	[37*]
Related null mutants		
TIMP-1	Loss of TIMP-1 in transformed cell lines can either potentiate or suppress frequency of tumor invasion	[26]
Point mutation	Marked dermal fibrosis	[51]
(cleavage site in type I collagen)	Impaired post partum uterine involution	-
u-PA	MMP-13 N-terminal cleavage accounts for bone resorption during embryonic and early adult life	
	Unable to activate pro-MMPs	[13*]
	On ApoE ^{-/-} background, protected from atherosclerotic macrophage infiltration and microaneurysm formation	

Pathological processes

Cardiovascular disease

Atherosclerosis is a chronic inflammatory process whereby plaques are formed in the intimal layer of the vessel wall as a result of accumulation of ECM, smooth muscle cells, and lipid-laden macrophages. In humans, coronary artery plaques may become unstable and rupture, triggering intravascular thrombosis leading to myocardial infarction. The atherosclerotic vessel wall may also dilate as a result of destruction of the medial elastic lamina, leading to aneurysm formation and rupture of the weakened vessel wall. Recently, plasminogen activators and several MMPs have been detected in association with human atherosclerotic arteries [10] and abdominal aortic aneurysms [11].

Mice with a targeted disruption of the apolipoprotein E (ApoE) gene have a delayed clearance of lipoproteins from the blood. When mice are fed a Western diet serum cholesterol levels reach 1400–2000 mg/dL and fatty streaks progressing to fibrous plaques develop at branch points of major vessels. The formation of these lesions is associated with macrophage recruitment causing disruption of the medial elastic lamina and microaneurysm formation. Complex lesions with plaque rupture and hemorrhage have yet to be observed for any model of atherosclerosis in the mouse (for a review see [12]).

To investigate the role of plasminogen activators (tissue-type plasminogen activators [t-PA] and urokinase-type plasminogen activators [u-PA]), Carmeliet and colleagues [13*] crossed ApoE^{-/-} mice with u-PA^{-/-} or t-PA^{-/-} mice. ApoE^{-/-} × u-PA^{-/-} mice, but not ApoE^{-/-} or ApoE^{-/-} × t-PA^{-/-} mice, were protected from macrophage-mediated destruction of medial elastic lamina and microaneurysm formation. Macrophages lined up along elastic lamina but they did not penetrate or disrupt these matrix structures. Because the ability of macrophages to degrade and migrate

through elastin is more likely due to macrophage elastase (MMP-12) [14] than to non-ecastolytic plasmin, these findings suggest that plasmin may activate MMP pro-enzymes, an old hypothesis not previously demonstrated *in vivo*. Indeed, in the absence of u-PA, macrophages were unable to convert macrophage pro-MMPs (MMP-3, MMP-9, MMP-12, and MMP-13) into their active forms in a reconstituted system [13*].

In contrast, in the human stromelysin-1 (MMP-3) promoter, a genetic polymorphism which causes diminished stromelysin-1 expression is associated with enhanced progression of atherosclerosis [15]. Together these and other studies suggest that MMPs initially maintain patency of the atherosclerotic vascular lumen at the risk of subsequent plaque rupture.

Cancer

MMPs are believed to promote tumor progression by initiating carcinogenesis, enhancing tumor angiogenesis, disrupting local tissue architecture to allow tumor growth, and breaking down basement membrane barriers for metastatic spread [16,17]. While some MMPs, such as matrilysin (MMP-7), collagenase-3 (MMP-13), and often gelatinase A (MMP-2), are expressed by tumor cells themselves, MMPs are predominantly produced by surrounding host stromal and inflammatory cells in response to factors released by tumors [17,18*]. MMPs may then bind to tumor cells and angiogenic endothelial cells, advancing tumor progression. For example, MMP-2 binds through its carboxy-terminal domain to $\alpha\beta_3$ integrin on melanoma cells and angiogenic blood vessels, enhancing tumor growth [19]. Autolytic processing of MMP-2 with release of the carboxy-terminal domain competes with cell surface binding of the enzyme, inhibiting angiogenesis and tumor growth [20*]. Consistent with these results, MMP-2^{-/-} host mice exhibit impaired primary tumor growth and

decreased experimental metastases of B16-BL6 melanoma and Lewis lung cell carcinoma (LLC) cells [21].

Matrilysin (MMP-7) is expressed by tumor cells derived from gastrointestinal epithelium, including those that arise spontaneously in mice with the adenomatous polyposis coli (APC) multiple intestinal neoplasia mutation *APC^{+/+}*. *MMP-7^{-/-} × APC^{+/+}* mice had delayed tumor development [22^{*}]. Similarly, stromelysin-3 deficient (*MMP-11^{-/-}*) mice demonstrated impaired tumor formation in response to chemical mutagenesis [23^{*}]. These studies confirm a role for MMPs in carcinogenesis. Potentially, ectopic expression of these proteinases (in combination with another mutation) may release cells from ECM-mediated cell cycle arrest. Alternatively, proteinases may release growth or angiogenesis factors promoting tumorigenesis.

While overexpression of tissue inhibitors of metalloproteinases (TIMPs), either in transgenic mice or by gene transfer, can decrease tumor progression in animal models [24,25], it has been difficult to demonstrate that lack of TIMP-1 in tumor or host consistently enhances tumor growth [26]. Perhaps expression of TIMPs 2-4 compensate for loss TIMP-1 expression in these models.

While MMPs commonly facilitate tumor progression, proteolytic cleavage products of MMPs may inhibit angiogenesis, limiting tumor progression. This was first apparent with the isolation of angiostatin from the urine of mice with LLC cells [27]. Angiostatin, a plasminogen cleavage product containing kringle domains 1-4, inhibits endothelial cell proliferation and is believed to be responsible for maintaining LLC lung metastases in a dormant state [27].

Generation of angiostatin in primary LLC tumors correlated with the presence of macrophages and macrophage elastase (MMP-12) [28]. The importance of MMP-12 in limiting lung metastasis growth in the LLC model has been confirmed by use of mice rendered deficient in macrophage elastase (*MMP-12^{-/-}*) by gene targeting (JL Gonsky and SD Shapiro, unpublished data). Preliminary studies suggest, however, that local expression of MMP-12 in macrophages surrounding the secondary lung metastases limits growth, in part through generation of angiostatin. This effect may also be related to MMP-2 processing by MMP-12 or other mechanisms.

Several other MMPs are also capable of generating angiostatin and other antiangiogenic fragments of plasminogen [29,30]. The kringle 5 domain by itself appears to have the greatest capacity to inhibit endothelial cell proliferation [31]. Serine proteinases, including plasmin, in association with an extracellular reductase that reduces disulfide bonds in plasmin, also trigger generation of angiostatin [32^{*},33]. In addition to angiostatin, other proteolytic fragments, most prominently endostatin (a 20 kDa carboxy-terminal fragment of type XVIII collagen), effec-

tively inhibit tumor angiogenesis [34^{*}]. In fact, treatment of several tumors in mice with endostatin resulted in prolonged tumor dormancy [35^{*}].

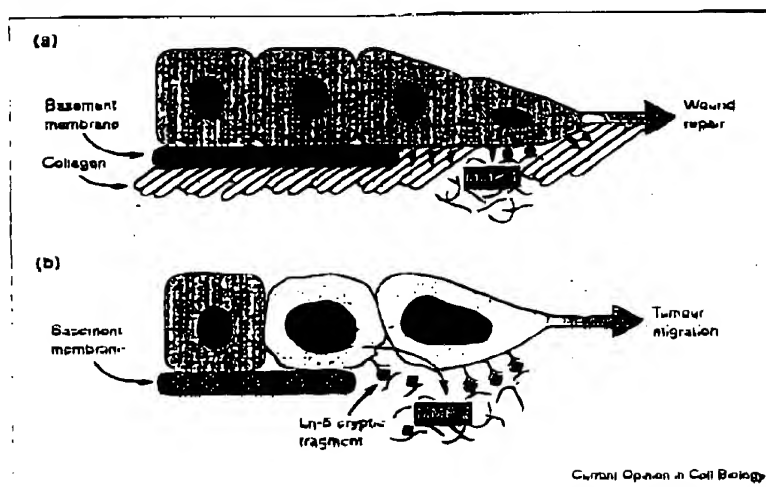
Thus, proteinases may benefit the host or the tumor depending upon spatial expression, proteolytic capacity, and binding affinity for matrix and tumor cells. Nevertheless, hydroxamates, which are MMP zinc-chelating agents, are effective in inhibiting growth of several primary tumors and metastases in animal models [1^{*}] including LLC [36]. The ability of these compounds to inhibit growth of neoplasms suggests that tumors use MMPs more effectively than the host; when all the MMPs are inhibited the host has the advantage. MMP inhibition combined with anti-angiogenic agents, such as angiostatin, endostatin, or $\alpha v \beta 3$ integrin, might prove optimal in clinical treatment of particular tumors.

Pulmonary emphysema

A major component of chronic obstructive pulmonary disease is destruction and enlargement of peripheral airspaces of the lung. Chronic exposure to cigarette smoke exposure leads to inflammatory cell recruitment and activation with release of elastases, in excess of inhibitors. ECM degradation coupled with abnormal repair results in lung destruction characteristic of emphysema. The serine proteinase neutrophil elastase is responsible for emphysema in patients with a genetic deficiency of its inhibitor α_1 -antitrypsin, a relatively uncommon form of the disease; however, the contribution of neutrophil elastase to the more common emphysema associated with cigarette smoking is controversial. It is possible that other neutrophil proteinases or enzymes from the more abundant macrophages contribute to lung damage associated with prolonged cigarette smoking.

Long-term exposure of wild-type (*MMP-12^{+/+}*) mice to cigarette smoke led to inflammatory cell recruitment followed by alveolar space enlargement similar to the pathologic defect in humans. Mice deficient in macrophage elastase (*MMP-12^{-/-}*), however, were protected from development of emphysema despite heavy long-term exposure to smoke. Surprisingly, *MMP-12^{-/-}* mice also failed to recruit monocytes into their lungs in response to cigarette smoke [37^{*}]. Because MMP-12 and most other MMPs are only expressed upon differentiation of monocytes to macrophages, it appeared unlikely that monocytes require MMP-12 for transvascular migration. More likely, cigarette smoke induces constitutive macrophages, which are present in lungs of *MMP-12^{-/-}* mice, to produce MMP-12 that in turn cleaves elastin, thereby generating fragments chemotactic for monocytes (JP Paige and SD Shapiro, unpublished data). This positive feedback loop would perpetuate macrophage accumulation and lung destruction. The concept that proteolytically generated elastin fragments mediate monocyte chemotaxis was first shown more than a decade ago [38-40].

Figure 1



ECM-mediated cell migration. (a) Keratinocyte migration during normal wound healing. Exposure of keratinocytes to interstitial collagen in the provisional matrix leads to high affinity binding (arrows) of $\alpha2\beta1$ integrin to collagen. This leads to expression of MMP-1, which degrades collagen and frees the cell to migrate, perhaps using other receptors (circles). $\alpha2\beta1$ binding to collagen at the migrating front 'pulls' the cell forward leading to re-epithelialization (adapted from [1]). (b) Epithelium-derived tumor cell migration. Tumor cells (lightly shaded) express MMP-2 (or induce stromal cells to produce it), which degrades the basement membrane (wavy lines), exposing cryptic fragments of laminin and collagen which are only exposed upon degradation (diamonds). These bind to cell receptors, leading to migration perhaps related to integrin-mediated signaling leading to cytoskeletal changes causing cell movement. LNS, laminin 5.

Bullous pemphigoid

The autoimmune subepidermal blistering disease known as bullous pemphigoid is characterized by deposition of autoantibodies at the basement membrane zone. In an experimental model of this disease in mice, the blistering is mediated by antibodies directed against the hemidesmosomal protein BP180 (collagen XVII), and depends on complement activation and neutrophil infiltration. In contrast to wild-type littermates, MMP-9^{-/-} mice were resistant to the blistering effects in this model despite deposition of autoantibodies and neutrophil recruitment equivalent to that seen in wild-type mice [41]. Whether MMP-9 directly causes blistering or augments neutrophil elastase activity by degrading $\alpha1$ -antitrypsin is currently unknown.

Bioactivity of ECM fragments

Proteolytically generated ECM (and non-ECM) fragments have long been thought to regulate a diverse array of processes in cell biology. The importance of this mode of regulation has been a recurrent theme in the recent *in vivo* studies presented here: plasmin mediates MMP activation, plasminogen fragments (angiotensin and other kringle domains) and collagen XVIII fragments (endostatin) inhibit neovascularization, while MMP-9 induces angiogenesis. Elastin fragments may regulate monocyte recruitment in chronic inflammation.

Overexpression of stromelysin-1 (MMP-3) in mammary glands of transgenic mice not only confirmed the expected role of MMPs in mammary gland branching morphogenesis, but unexpectedly demonstrated an additional role in regulating post-partum mammary gland involution [42]. A series of subsequent studies demonstrated that cell contact with correct tissue architecture is crucial for cell homeostasis,

suppression of apoptosis, and maintenance of differentiated phenotype (see N Boudreau and MJ Bissell, pp 640-646).

It was also recently recognized that cleavage of the laminin-5 $\gamma2$ chain by gelatinase A (MMP-2) exposed a cryptic site within laminin, inducing migration of malignant breast epithelial cells [43]. This study suggests that local proteinase concentration may determine cell behavior. Proteolysis is required to initiate and sustain migration, but excessive proteolysis may degrade matrix signals and receptors, thereby disrupting cell matrix interactions and inhibiting migration [1]. With respect to epithelial cell migration in normal wound healing, Parks and co-workers [44] hypothesized that interaction of keratinocyte $\alpha2\beta1$ integrin with native type I collagen in a provisional wound matrix induces collagenase-1 (MMP-1) expression. By cleaving collagen, the initial high affinity contact is loosened, releasing the cell, which then migrates to 'grab' high affinity $\alpha2\beta1$ integrin bonds with undigested collagen ahead in the open wound (Figure 1). Indeed, keratinocytes can migrate on native collagen but not on a collagenase-resistant collagen matrix [45]. Similarly, in fibroblasts, binding of fibronectin fragments (but not intact fibronectin) to $\alpha5\beta1$ integrin signals activator protein-1 (AP-1)-mediated induction of MMP-1 synthesis [46]. Thus, ECM provides an important mechanism for cells to communicate with their external environment. When cells are in contact with their appropriate, intact ECM they are quiescent (or at least perform their normal functions); however, cell contact with inappropriate, altered, or disrupted ECM sets in motion a variety of signal transduction pathways and gene transcription resulting in many cellular responses with the goal of tissue repair [47].

In addition to MMPs, closely related metalloproteinases termed ADAMs (a disintegrin and metalloproteinase

domain) are well positioned on the cell surface to release or 'shed' a variety of important inflammatory cell mediators (see RA Black and JK White, pp 654-659). Earlier it was discovered that hydroxamic acid MMP inhibitors prevented release of latent tumor necrosis factor α (TNF) from monocyte surfaces, and subsequently the gene encoding the responsible proteinase, TACE (TNF convertase), or ADAM-18, was cloned. Metalloproteinases also mediate shedding of L-selectin, IL-6, Fas, TNF receptor, and a variety of other TNF receptor superfamily members. Additionally, significant stores of matrix bound transforming growth factor β may also be proteolytically released by plasmin and perhaps MMPs [48].

Conclusion and future prospects

Why are there so many MMPs? Overlapping but distinct substrate specificities and cell-specific expression suggest potentially unique functions, but clearly a variety of MMPs are expressed during development, perhaps to compensate for potential loss of an individual MMP. This diversity would explain why the phenotypes of MMP-mutant mice have been so mild with respect to development and other physiologic processes. Alternatively, MMPs may have a primary function in repair and defense, and in mature tissues, rather than a requisite function in morphogenesis. Further developmental analyses and mice with multiple MMP deficiencies will help address this issue. In contrast, aberrant or excessive expression of individual MMPs causes certain destructive diseases. Consequently, it has been easier to show that deletion of specific, abnormally expressed MMPs prevents disease onset. Greater understanding of similarities between humans and mice will guide rational medical therapy in the future. Gene-targeted mice will help investigators dissect molecular pathways and further define the role of proteolytic ECM (and non-ECM) cleavage products as regulators of gene transcription, angiogenesis, cell migration, inflammation, and cell cycle control, independent of translational research aspects.

Acknowledgements

This work was supported by the National Institutes of Health and by the Alan A and Edith Wolf Charitable Trust.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Parke WC, Mecham RP (Eds): *Matrix metalloproteinases*. San Diego: Academic Press; 1998. Comprehensive and up-to-date reviews on many aspects of MMP biology and chemistry.
 2. Brown PD: Matrix metalloproteinase inhibitors in the treatment of cancer. *Med Oncol* 1997; 14:1-10.
 3. Piaton K: A miracle enough: the power of mice. *Nat Med* 1995; 1:215-220.
 4. Shapiro SD: Mighty mice: Transgenic technology 'knocks out' questions of matrix metalloproteinase function. *Matrix Biol* 1997; 15:527-533.

5. Olson EN, Arnold M-H, Rigby PWJ, Weis BJ: Know your neighbors: three phenotypes in null mutants of the myogenic bHLH gene *Myf4*. *Cell* 1998; 95:1-4.
6. Hawley DL, Rudolph LA, Marmè LM: Matrix metalloproteinases as mediators of reproductive function. *Mol Hum Reproduction* 1997; 3:27-45.
7. Wu TH, Shipley JM, Bergers G, Berger JE, Helms JA, Hanahan D, Shapiro SD, Senior RM, Werb Z: MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. *Cell* 1998; 93:411-422.
- This initial description of MMP-9-deficient mice demonstrates the role of MMP-9 in long bone development. MMP-9 induces primary angiogenesis associated with vascular invasion of the cartilage matrix that precedes endochondral ossification.
8. Leininger B, Trugnan G, Murphy G, Ronce PM: Matrix metalloproteinases MMP2 and MMP9 are produced in early stages of kidney morphogenesis but only MMP9 is required for renal organogenesis *in vivo*. *J Cell Biol* 1997; 138:1383-1373.
9. Minor JM, Behrman T, Shipley JM, Senior RM: Renal function is normal in gelatinase B deficient mice [abstract]. *Mol Biol Cell* 1997; 8:403.
10. Libby P: Molecular bases of the acute coronary syndromes. *Circulation* 1995; 91:2844-2850.
11. Thompson RW, Mertens RA, Liao S, Holmes DR, Mecham RP, Weigus HG, Parke WC: Production and localization of 92-kD gelatinase in abdominal aortic aneurysms: an elastolytic metalloproteinase expressed by aneurysm-infiltrating macrophages. *J Clin Invest* 1995; 96:318-328.
12. Breslow J: Mouse models of atherosclerosis. *Science* 1996; 272:685-688.
13. Carmeliet P, Moons L, Lijnen R, Crawley J, Tipping P, Drou A, Eschke H, Shapiro SD, Luyck F, Collen D: Plasmin predisposes to atherosclerotic aneurysm formation by activation of matrix metalloproteinases. *Nat Genet* 1997; 17:438-444.
- ApoE^{-/-} mice also lacking urokinase-type (but not tissue-type) plasminogen activators are protected from macrophage penetration of elastic lamina and microaneurysm formation. This paper contains the most direct evidence to date that plasmin generated by urokinase-type plasminogen activators is required for pro-MMP activation *in vivo*.
14. Shipley JM, Wenzel-Schmidt RL, Kobayashi DK, Loy T, Shapiro SD: Metalloelastase is required for macrophage-mediated proteolysis and matrix invasion in mice. *Proc Natl Acad Sci USA* 1996; 93:3942-3946.
15. Ye S, Eriksson P, Hamsten A, Kurhonen M, Humphries SE, Menzies AM: Progression of coronary atherosclerosis is associated with a common genetic variant of the human stomelysin-1 promoter which results in reduced gene expression. *J Biol Chem* 1996; 271:13055-13060.
16. Coussens LM, Werb Z: Matrix metalloproteinases and the development of cancer. *Chem Biol* 1996; 3:895-904.
17. Powell WC, Marmè LM: Complex roles of matrix metalloproteinases in tumor progression. *Curr Top Microbiol Immunol* 1996; 213:1-21.
18. Guo H, Zucker S, Gordon MK, Toole BP, Bissas C: Stimulation of matrix metalloproteinase production by recombinant extracellular matrix metalloproteinase inducer from transfected Chinese hamster ovary cells. *J Biol Chem* 1997; 272:24-27.
- Previously this group found that extracellular matrix metalloproteinase inducer (EMMPRIN), a transmembrane glycoprotein attached to the surface of many types of malignant human tumor cells, stimulated matrix metalloproteinase (MMP) synthesis in fibroblasts. This paper, continuing Bissas' pioneering studies post-mortem, shows that CHO cells transfected with EMMPRIN post-translationally process the molecule and the stimulates human fibroblast production of MMPs-1, -2, and -3.
19. Brooks PC, Stetler-Stevenson WG, Quigley JP, Chiriac DA: Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin α 5 β 1. *Cell* 1998; 95:683-693.
20. Brooks PC, Stetler-Stevenson WG, Quigley JP, Chiriac DA: Disruption of angiogenesis by PEX, a noncatalytic metalloproteinase fragment with integrin binding activity. *Cell* 1998; 92:391-400.
- Data in [17] demonstrated that MMP-2 binds to tumor cell α 5 β 1 integrin and confirmed the relationship of this event to tumor progression. This report takes us further showing that dissociation of the carboxyterminal

- domain from the catalytic domain occurs *in vivo* and the free carboxyl terminus both activates pro-MMP-2 and competes with MMP-2 tumor cell binding, inhibiting growth.
21. Hah T, Tanaka M, Yoshida M, Yoshoka T, Nishimoto M, Kohara S: Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. *Cancer Res* 1998, 58:1048-1051.
 22. Wilson CL, Hopper KJ, Labosky PA, Hagan BL, Mariani LM: Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin. *Proc Natl Acad Sci USA* 1997, 94:1402-1407.
- This report and [21], utilizing gene-targeted mice, provide evidence for involvement of matrix metalloproteinases directly in the process of carcinogenesis, rather than merely "clearing the way" for tumors to move.
23. Masson R, Lefebvre O, Noel A, El Fahime M, Chénard M-P, Wenzel C, Kebers F, LeMaur M, Diehl A, Foidart J-M et al: *In vivo* evidence that the serine protease-3 metalloproteinase contributes in a permissive manner to epithelial cell malignancy. *J Cell Biol* 1998, 144:1535-1541.
- See annotation to [20].
24. Martin DC, Rother U, Sanchez-Sweatman OM, Orr FW, Khosla R: Inhibition of SV40 T antigen-induced hepatocellular carcinoma in TIMP-1 transgenic mice. *Oncogene* 1998, 13:569-576.
 25. Imren S, Kohn DB, Shimada M, Blauer L, DeClerck YA: Overexpression of tissue inhibitor of metalloproteinases-2 (TIMP-2) inhibits tumor growth *in vivo* and inhibits tumor growth and invasion. *Cancer Res* 1998, 58:2881-2885.
 26. Seidman PD, Alexander CM, Werb Z, Jaenisch R: Targeted mutagenesis of TIMP-1 reveals that lung tumor invasion is influenced by TIMP-1 genotype of the tumor but not by that of the host. *Oncogene* 1998, 13:2307-2314.
 27. O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenblatt RA, Moses M, Lane WS, Cao Y, Sage EH, Folkman J: A novel angiogenesis inhibitor which mediates the suppression of metastasis by a Lewis lung carcinoma. *Cell* 1994, 79:315-328.
 28. Dong Z, Kumar R, Yang X, Fidler IJ: Macrophage-derived metalloproteinase is responsible for the generation of angiostatin in Lewis lung carcinoma. *Cell* 1997, 88:801-810.
- This report provided the first evidence for proteolytic generation of angiostatin from plasminogen. Expression of macrophage elastase correlated with angiostatin generation in Lewis lung carcinoma primary tumors.
29. Patterson BC, Sang QA: Angiotensin-converting enzyme activities of human matrilysin (MMP-7) and gelatinase B/type IV collagenase (MMP-9). *J Biol Chem* 1997, 272:28823-28826.
 30. Cornelius LA, Nanning L, Klein B, Pierce R, Bohinski M, Welgus HG, Shapiro SD: Generation of angiostatin by matrix metalloproteinases: effects on neovascularization. *J Immunol* 1998, in press.
 31. Cao Y, Chen A, An SSA, Ji RW, Davidson D, Linza M, Kringle 5 of plasminogen is a novel inhibitor of endothelial cell growth. *J Biol Chem* 1997, 272:22224-22228.
 32. Stetler-Stevenson MG, Stetler-Stevenson MG, Stetler-Stevenson MG, Stetler-Stevenson MG: Generation of angiostatin by reduction and proteolysis of plasmin: catalysis by a plasminogen activator secreted by cultured cells. *J Biol Chem* 1997, 272:20641-20645.
- This paper not only demonstrates the capacity of reduced plasmin to generate angiostatin from its parent plasminogen, but also gives the first example of an extracellular reduction of a protein disulfide bond.
33. Gargy S, Twardowski SP, Stack MS, Patrick M, Boggio L, Cundiff DL, Schnaper HW, Madison L, Volpert O, Bovee N et al: Human prostate carcinoma cells express enzymatic activity that converts human plasminogen to the angiogenesis inhibitor, angiostatin. *Cancer Res* 1996, 56:4887-4892.
 34. O'Reilly M, Boehm T, Shing Y, Fukui N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR, Folkman J: Endostatin: An endogenous inhibitor of angiogenesis and tumor growth. *Cell* 1997, 88:277-285.
- Continuing the theme that protein fragments inhibit angiogenesis, endostatin, a 20 kDa carboxy-terminal fragment of collagen XVIII, was identified from hemangioendotheliomas. Endostatin inhibited endothelial cell proliferation *in vitro* and angiogenesis and tumor growth *in vivo*.
35. Boehm T, Folkman J, Browder T, O'Reilly MS: Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. *Nature* 1997, 390:404-407.
- Treatment of Lewis lung cell carcinomas, T241 fibrosarcomas, and B16 melanomas in mice with endostatin resulted in prolonged tumor dormancy.
- No drug resistance occurred and there was no tumor recurrence after several cycles of repeated therapy.
36. Anderson IC, Shipp MA, Docherty AJP, Teicher BA: Combination therapy including a gelatinase inhibitor and cytotoxic agents reduces local invasion and metastasis of murine Lewis lung carcinoma. *Cancer Res* 1998, 58:710-715.
 37. Hanjani RD, Kobayashi DK, Senior RM, Shapiro SD: Macrophage elastase is required for cigarette smoke-induced emphysema in mice. *Science* 1997, 277:2002-2004.
- This study supports the 35 year old elastase/alpha1-antitrypsin hypothesis for the pathogenesis of emphysema. Whether macrophage elastase plays a significant role in the human disease is less certain. Yet, MMP-12^{-/-} mice provide a model for macrophage proteolysis, demonstrating a critical role of macrophages in emphysema and unmasking a protease-dependent mechanism of inflammatory cell recruitment.
38. Senior RM, Griffin GL, Mecham RP: Chemotactic activity of elastin-derived peptides. *J Cell Invest* 1980, 66:859-862.
 39. Hunninghake GW, Davidson JM, Rappaport S, Scapellato S, Gadek JE, Crystal RG: Elastin fragments attract macrophage precursors to diseased sites in pulmonary emphysema. *Science* 1981, 212:925-927.
 40. Senior RM, Griffin GL, Mecham RP, Wharm DS, Prasad KV, Urry DW: Val-Gly-Val-Ala-Pro-Gly, a repeating peptide in elastin, is chemotactic for fibroblasts and monocytes. *J Cell Biol* 1984, 99:870-874.
 41. Liu Z, Shipley JM, Vu T, Zhou X, Diaz LA, Werb Z, Senior RM: Gelatinase B-deficient mice are resistant to experimental bullous pemphigoid. *J Exp Med* 1998, in press.
 42. Sympton CJ, Taffourh RS, Alexander CM, Chen JR, Chen SM, Russell MJ: Targeted expression of stromelysin-1 in mammary gland provides for a role of proteinases in branching morphogenesis and the requirement for an intact basement membrane for tissue-specific gene expression. *J Cell Biol* 1996, 132:581-593.
 43. Giannelli G, Fabbro-Marzeller J, Sorensen O, Stetler-Stevenson MG, Quaranta V: Induction of cell migration by matrix metalloproteinase-2 cleavage of laminin-5. *Science* 1997, 277:325-328.
- This article demonstrates the existence of promitogenic sites of laminin specific for gelatinase A. This study and [1] suggest that local proteinase concentration and matrix degradation determine cell behavior.
44. Sudbeck BD, Picher BK, Welgus HG, Parks WC: Induction and repression of collagenase-1 by keratinocytes is controlled by distinct components of different extracellular matrix compartments. *J Biol Chem* 1997, 272:22103-22110.
- See annotation to [46].
45. Picher BK, Dunn JA, Sudbeck BD, Krane SM, Welgus HG, Parks WC: The activity of collagenase-1 is required for keratinocyte migration on a type I collagen matrix. *J Cell Biol* 1997, 137:1445-1457.
- This reference and [44] build a story of how keratinocyte interaction with collagen in a provisional wound matrix initiates reepithelialization.
46. Tremble P, Damay C, Werb Z: Components of the nuclear signaling cascade that regulates collagenase gene expression in response to integrin-derived signals. *J Cell Biol* 1995, 129:1707-1720.
 47. Werb Z: ECM and cell surface proteolysis: Regulating cellular ecology. *Cell* 1997, 91:439-442.
- This is an excellent review highlighting how proteolysis of matrix at the cell surface influences a variety of cell functions.
48. Munger JS, Hargrett-Scott N, Ghossein PE, Mazzieri R, Nunes J, Rifkin BR: Latent transforming growth factor-beta: structural features and mechanisms of activation. *Kidney Int* 1997, 51:1378-1382.
 49. Ren T, Kudo T, Gomi M, Nakao S, Suzuki T, Kohara S: Unaltered secretion of beta-trypsin precursor protein in gelatinase A (MMP-2)-deficient mice. *J Biol Chem* 1997, 272:22389-22392.
 50. Mudgett JS, Hutchinson NI, Chantana NA, Forsyth AJ, McDonnell J, Singhal IL, Byrne EK, Ransig J, Kunkel D, Shen CP et al: Susceptibility of stromelysin 1-deficient mice to collagen-induced arthritis and cartilage destruction. *Arthritis Rheum* 1998, 41:110-121.
 51. Lu X, Wu H, Byrne M, Jeffrey J, Krane S, Jaenisch R: A targeted mutation at the known collagenase cleavage site in mouse type I collagen impairs tissue remodeling. *J Cell Biol* 1995, 130:227-237.

83. Loureiro J, Peifer M. Roles of Armadillo, a *Drosophila* catenin, during central nervous system development. *Curr Biol* 1998; 8:622-632.
- The study reports the discovery of a new isoform of armadillo in *Drosophila*, containing an alternatively spliced carboxy-terminal domain that plays a role in the development of the central nervous system of *Drosophila*.
84. Munn PJ, Sparks AB, Konnek V, Barker N, Clevers H, Vogelstein B, Kinzler KW. Activation of β -catenin Tcf-signaling in colon cancer by mutations in β -catenin or APC. *Science* 1997; 275:1787-1790.
- This study demonstrates that in certain colon carcinoma cell lines the level of β -catenin is elevated owing to mutations on amino-terminal serine residues of β -catenin which are important for regulating its degradation. Such mutant β -catenin molecules are insensitive to adenomatous polyposis coli (APC)-directed degradation of β -catenin and the transcription driven by the lymphoid enhancer binding factor (LEF)/T-cell specific factor (TCF)- β -catenin complex in such cell lines is not inhibited by transfected APC.
85. Konnek V, Backer NP, Munn J, van Wichen D, de Weger R, Kinzler KW, Vogelstein B, Clevers H. Constitutive transcriptional activation by a β -catenin-Tcf complex in APC^{-/-} colon carcinoma. *Science* 1997; 275:1784-1787.
- This study shows that there is constitutive β -catenin-driven LEF/TCF-dependent transcription in adenomatous polyposis coli (APC)^{-/-} colon carcinoma cell lines and transfection of wild-type APC can block this transcriptional.
86. Rubinfeld B, Robbins P, El-Gamil M, Albert I, Porfir E, Polakis P. Stabilization of β -catenin by genetic defects in melanoma cell lines. *Science* 1997; 275:1790-1792.
- This is the first demonstration in human melanoma of increased β -catenin levels owing either to mutations in the adenomatous polyposis coli (APC) gene or to mutations in the amino-terminal serine residues of β -catenin that are phosphorylated by glycogen synthase kinase 3 β and that are important for the regulation of β -catenin degradation. In addition, these cells were shown to contain constitutive β -catenin-lymphoid enhancer binding factor (LEF)-1 complexes, suggesting that these are involved in tumor progression in melanoma.
87. Peifer M. β -catenin as oncogene: the smoking gun. *Science* 1997; 275:1752-1753.
- This is a very attractively written review on the important breakthrough processes in [84th-86th] suggesting that β -catenin can act as an oncogene.
88. Tsukamoto AS, Grosschedl R, Guzman RC, Parslow T, Varmus HE. Expression of the Int-1 gene in transgenic mice is associated with mammary gland hyperplasia and adenocarcinomas in male and female mice. *Cell* 1988; 55:619-626.
89. Whitchead I, Kirk M, Kay R. Expression cloning of oncogenes by retroviral transfer of cDNA libraries. *Mol Cell Biol* 1996; 15:704-710.
90. Rubinfeld B, Albert I, Porfir E, Maramba S, Polakis P. Loss of β -catenin regulation by the APC tumor suppressor protein correlates with loss of structure due to common somatic mutations of the gene. *Cancer Res* 1997; 57:4624-4630.
- This study demonstrates that the somatic mutations in adenomatous polyposis coli (APC) seen in human colon cancers that are clustered in a very narrow region of the gene are localized on the APC molecule in a domain that is responsible for regulating the binding and degradation of β -catenin, suggesting that this site is selected for during tumor progression.
91. Sparks AB, Munn PJ, Vogelstein B, Kinzler KW. Mutational analysis of the APC/ β -catenin/Tcf pathway in colorectal cancer. *Cancer Res* 1998; 58:1130-1134.
- In this study, the authors searched for possible mutations in the genes for β -catenin, adenomatous polyposis coli (APC) and other components of the Wnt pathway in colon cancers, and found mutations in either β -catenin or APC, but not other known components of the pathway. These mutations were mutually exclusive (either in β -catenin or in APC) and thus equivalent, since both affect the stability of β -catenin. This further supports the role of changes in β -catenin stability in colon cancer.
92. Zurawski RH, Chippa SA, Allen C, Raffaeli C. Sporadic medulloblastomas contain oncogenic β -catenin mutations. *Cancer Res* 1998; 58:898-899.
- See annotation to [84th].
93. Pajcic J, Gamallo C. Mutations in the β -catenin gene (CTNNB1) in endometroid ovarian carcinoma. *Cancer Res* 1998; 58:1344-1347.
- See annotation to [84th].
94. Takahashi M, Fukuda K, Sugimura T, Wakabayashi K. β -catenin is frequently mutated and demonstrates altered cellular location in azoxymethane-induced rat colon tumors. *Cancer Res* 1998; 58:42-46.
- This study and the ones in [92,93rd] describe mutations in the amino terminus of the β -catenin gene that have been shown to be responsible for regulating the stability of β -catenin in other types of cancer, in addition to colon cancer [84th,85th,91st] and melanoma [88th], suggesting that such mutations may play a role in tumor progression in a larger variety of human cancers than was previously recognized.
95. Abadie H, Bickham C, Torchard D, Serova O, Wagnen T, Nan E, Worching J, Hardtampfer C, Montagna M, Lynch HT, Lamer GM et al. The human plakoglobin gene localizes on chromosome 17q21 and is subject to loss of heterozygosity in breast and ovarian cancer. *Proc Natl Acad Sci USA* 1995; 92:6384-6388.
96. Vlamincx KL, Vahedi J, Marzouf M, Fiers W, Van Roy F. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. *Cell* 1991; 66:107-119.
97. Bullions LC, Notteman DA, Chung LS, Levine AJ. Expression of a wild type α -catenin protein in cells with a mutant α -catenin gene restores both growth regulation and tumor suppression activities. *Mol Cell Biol* 1997; 17:4501-4508.